Acta Neuropathol (Berl) (1985) 68:245–252

Acta Neuropathologica

Herpes Simplex Virus Antigen Detection in Human Acute Encephalitis:

An Immunohistochemical Study using Avidin-Biotin-Peroxidase Complex Method*

C. Charpin[1], D. Gambarelli[1], M. N. Lavaut[1], J.M. Seigneurin[2], M. Raphael[1], M. Berard[1], and M. Toga[1]

[1] Laboratoire de Neuropathologie, Faculté de Médecine Timone, 27, Bd. Jean Moulin, F-13385 Marseille Cédex 05, France
[2] Laboratoire de Bactériologie et de Virologie, CHU Grenoble, F-38043 Grenoble, France

Summary. Autopsy specimens from six patients with clinically diagnosed herpes simplex virus (HSV) encephalitis were studied. Since immunocytochemistry has been reported to be a more reliable and successful method to identify HSV as the etiologic agent, antitype 1 HSV (HSV1) and antitype 2 HSV (HSV2) and the avidin-biotin-peroxidase complex (ABC) method were applied to brain paraffin sections. Positive immunostaining was observed in front-orbital, mediobasal temporal lobes, cingulate gyrus, and insula. The staining was bilateral but predominant on one side. In neurons, the cytoplasmic staining was prominent in perikarya and processes, less often observed in nuclei, rarely seen in nuclear inclusions. The positive staining was intense in oligodendrocytes and macrophages, in both nuclei and cytoplasm. In two cases astrocytic processes were stained strongly. Perivascular lymphocytes were always negative. Positive reactions were obtained with both anti-HSV1 and anti-HSV2 but weaker with anti-HSV2. This results suggests that, because of its high sensitivity, ABC method permits viral antigen detection not feasible with other methods. However, this method lacks of accuracy for HSV typing mainly because of probable antigens changes resulting from tissue processing.

Key words: Immunoperoxidase – Herpes virus – encephalitis

Introduction

Clinical data and neuropathologic findings have been adequately described in the literature [5, 20, 24, 28] but do not give sufficient proof that herpes simplex virus is the etiologic agent for the acute necrotizing encephalitis. Since standard virologic methods often fail to isolate HSV in cerebrospinal fluid (CSF) or tissues [15], immunocytochemical procedure constitutes the more effective and reliable method for HSV antigen demonstration even on routinely fixed tissues and stored paraffin blocks. In the present study we investigated samples from six patients with acute HSV encephalitis, the diagnosis of which was evoked on a clinical basis: we used avidin-biotin-peroxidase complex method and rabbit anti-HSV1 and anti-HSV2 to (a) determine virus antigen localization in brain and distribution in cells and (b) attempt to proceed to antigen typing in damaged tissues.

Case Reports

Four patients were male and two female. Their ages ranged from 15 to 74 years. The onset of the neurologic disease was confusion in four cases (cases 2–5) associated with a meningeal syndrome in one case (case 4), or seizures in two cases (cases 1 and 6). In all the cases hyperthermia was associated with the early neurologic symptoms. Motor weakness, or focal paresis, or hemiparesis consistently followed the onset signs. Seizures appeared later in one case (case 2) and meningeal symptoms in four cases (cases 1, 3–5). In all the cases the patients progressed rapidly to stupor and coma with few clinical clues to suggest localization. The survival times were from 3 days to 30 days, less than 9 days in four cases.

Cerebrospinal fluid (CSF) showed a pleiocytosis ranging from 32 to 300 cells per mm^3 with 90% of lymphocytes. The CSF protein content was clearly elevated in two cases and slightly elevated in four. Herpes simplex virus (HSV) could not be identified in CSF, but was recovered in one case (case 3) from a postmortem brain biopsy. An electroencephalogram (EEG) was performed in five cases and showed characteristic slow wave complexes at regular intervals of two to three per second focus on the right side in three cases and on the left side in one case. The computerized tomography performed in two cases showed low density abnormalities in temporal lobes.

* Supported by the "Fondation pour la Recherche Médicale", "Conseil Scientifique de la Faculté de Médecine"
Offprint requests to: Dr. C. Charpin (address see above)

Table 1. Immunostaining (avidin-biotin-peroxidase complex) findings in herpes simplex virus (HSV) encephalitis with anti-type 1 HSV (HSV1) and anti-type 2 HSV (HSV2)

Cases	AC type	Neurons		Oligodendrocytes		Astrocytes	Macrophages	Lymphocytes	Leptomeninges
		Cytopl.	Nucl.	Cytopl.	Nucl.				
1	HSV1	− (necr. ++)	−	+++	+++	−	+++	−	−
	HSV2	−	−	++	++	−	+++	−	−
2	HSV1	+	−	++	++	−	++	−	−
	HSV2	+	−	+	+	−	+	−	−
3	HSV1	++++	+	+++	+++	−	+++	−	−
	HSV2	++++	+	++	++	−	++	−	−
4	HSV1	+++	++	++	++	+	++	−	−
	HSV2	+	−	+	+	−	+	−	−
5	HSV1	+++	+++	++++	++++	++	++++	−	−
	HSV2	+++	+++	++	++	+++	++	−	+ (macroph.)
6	HSV1	++++	++++	+++	+++	++	+++	−	++ (macroph.)
	HSV2	++	++	++	++	++++	+++	−	+ (macroph.)

Materials and Methods

Tissue Preparations

Autopsy specimens were fixed in 10% formalin for 4 weeks. Only grossly abnormal areas were sampled with adjacent brain tissue. Large sections of brain were embedded in paraffin blocks stored for up to 17 years. Large 10 μm-thick paraffin sections were deparaffinized with xylene and hydrated in graded concentrations of ethanol before staining with hemalun-eosin (HE) for standard light microscopy examination.

For immunostaining. smaller specimens were sampled to spare the antibodies: areas with prominent histological abnormalities were identified on paraffin blocks after HE study and small 5–6 μm-thick sections were mounted on small slides.

In two cases (cases 3 and 4) brain tissue from areas displaying numerous Cowdry type A inclusions were sampled for electron microscopy: formalin-fixed tissues were washed in cacodylate buffer for 3 days and postfixed in osmium tetroxide before araldite embedding.

Immunostaining Procedure

Antibodies. Specific rabbit anti-herpes simplex type 1 antibody, Mac-Intyre (HSV1) and anti-herpes simplex type 2 antibody, MS, (HSV2) were obtained from Dako Laoratories (Copenhagen, Denmark) and diluted 1:100. Monoclonal anti-HSV1 and monoclonal anti-HSV2 were supplied by the Institut Pasteur Production (France).

Immunoperoxidase staining. Immunoperoxidase technique was performed as described previously [5, 6, 13] using aviding-biotin-peroxidase complex (ABC kits: Vector Labs; Burlingame, CA, USA). According to this method the sections were first incubated with normal goat serum, then with rabbit or mouse anti-HSV1 and anti-HSV2. The sections were then incubated in biotin-labeled goat anti-rabbit or anti-mouse IgG antibody, and endogenous peroxidase acitivity was blocked with a 0.3% hydrogen peroxide solution for 20 min before applying avidin-biotin-peroxidase complex. The slides were subsequently stained in AEC (3-amino-9-ethyl-carbazole, Sigma), counterstained in Mayer's hemalun, and mounted in glycerinated (80%) gelatin (4%) solution. A + to ++++ grading system was used by three of us in an attempt to quantify the number of immunostained cells.

Controls. Negative controls were (a) phosphate-buffered saline (PBS) which eliminated nonspecific immunostaining due to the ABC kit reagents, (b) substitution of specific HSV antibodies by irrelevant anti-serum (i.e., anti-casein) to control nonspecific immunoreaction due to anti-HSV antibodies, (c) normal brain tissue devoid of histological encephalitis, and (d) brain tissue sections from non HSV encephalitis.

Results

Neuropathologic Findings

In all the cases morphological abnormalities were characteristic of HSV encephalitis. Cases 3, 4, and 5 have already been described morphologically by one of us [28]. Light microscopy study revealed multiple areas of necrosis mainly involving the cortex but visible also in the white matter in cases 5 and 6. Adjacent to this necrosis foci of inflammation with sheets of fatty and foamy macrophages, perivascular cuffings with lymphocytes and gliomesenchymal cells nodules were observed. Cowdry type A intranuclear inclusion bodies were located in neuronal and glial cells.

The distribution of this pathologic abnormality was characteristic of acute herpes necrotizing encephalitis: a bilateral involvement was observed with striking predominence in one side. Necrosis and inflammation were located mainly in basal fronto-orbital lobes and the mediobasal temporal lobes, cingular gyri, and insula.

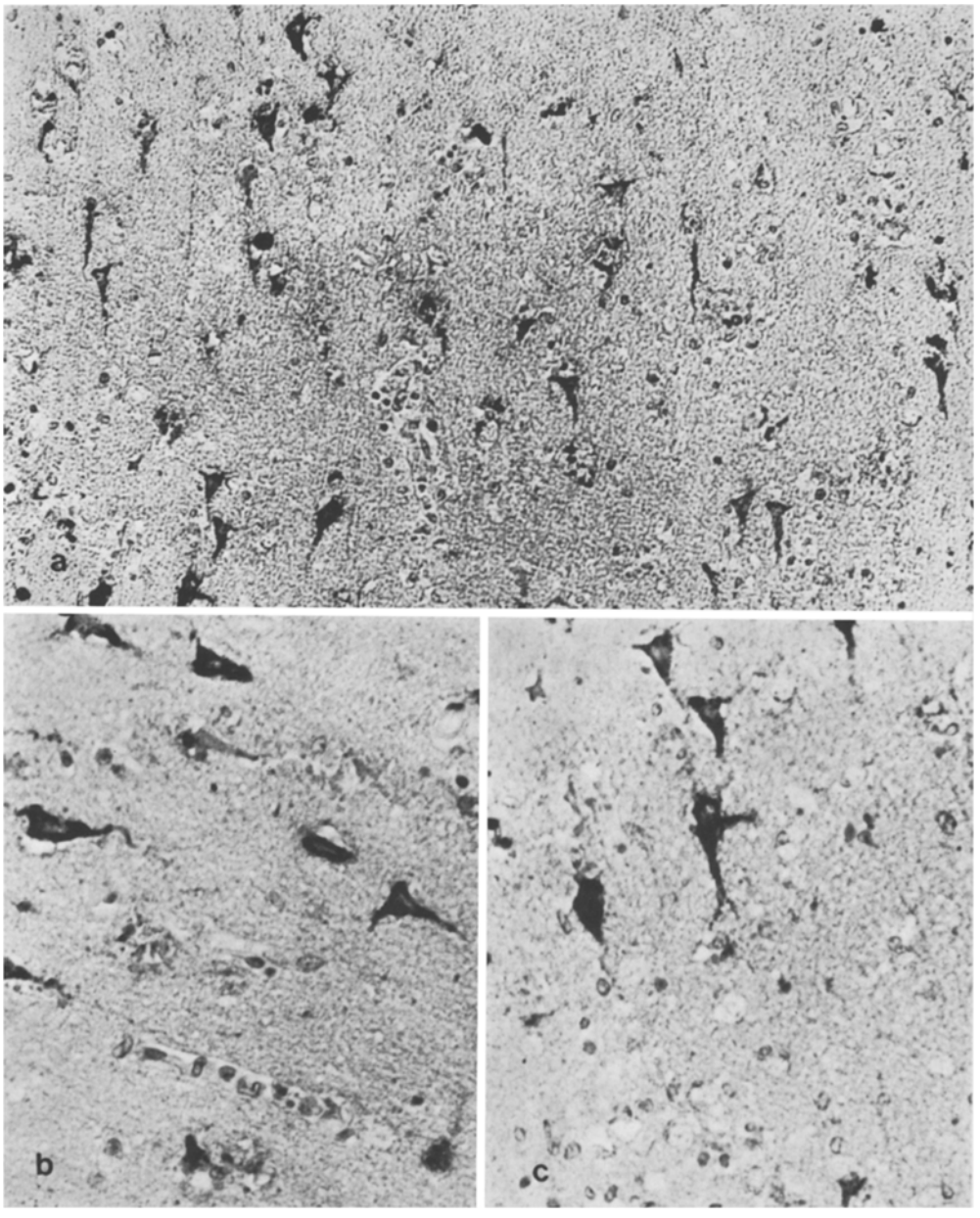

Fig. 1. a Strong positive staining with rabbit anti-type 1 herpes simplex virus in neurons of temporal lobe cortex in case 3 (avidin-biotin-peroxidase complex "ABC" immunostaining + counterstain, ×200). **b, c** Higher magnification showing a prominent cytoplasmic staining in perikarya and neuronal processes, whereas nuclei most often remain unstained (ABC immunoperoxidase + counterstain, ×320)

Immunocytochemical Findings (Table 1)

The positive immunoreactions consisted in typical finely granular reddish material located in both neuronal and glial cells. In three cases (cases 2–4) cortical neuronal involvement appeared more positively stained than white matter cells, that were strongly positive in cases 5 and 6. In case 1, due to a marked cortical necrosis, the positive staining in the white matter was predominent. When the encephalitis was severe the positive immunostaining in the white matter was enhanced.

Neuronal staining was more prominent in the cytoplasm although the nucleus was stained focally (Figs. 1, 2). Positive nuclear inclusions were rarely observed (Figs. 2, 3). Positive cytoplasmic staining was observed in both perikarya and neuronal processes (Fig. 1). In all the cases, foamy fatty cells and oligodendrocytes displayed positive staining mainly located in the nucleus less often in both nucleus and

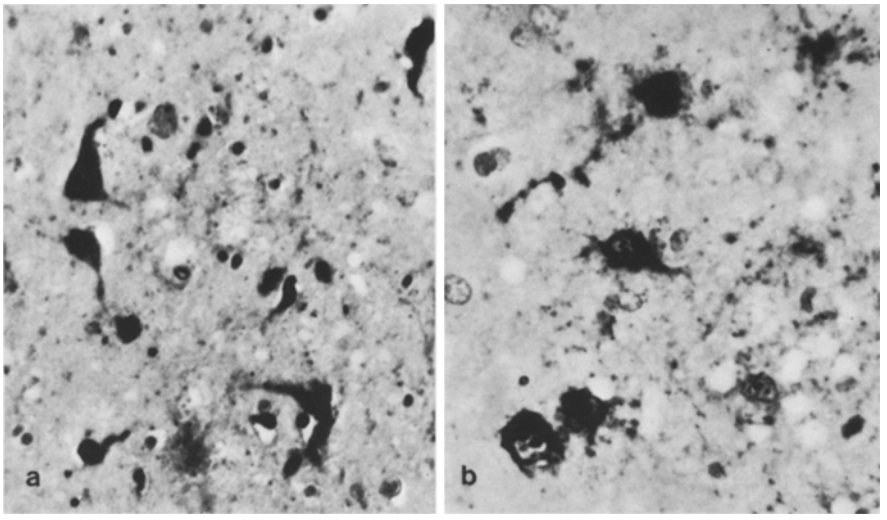

Fig. 2. a Positive anti-type 1 HSV immunostaining in both cytoplasm and nuclei of neurons and **b** in oligodendrocytes in case 1 (ABC immunoperoxidase + counterstain, ×380)

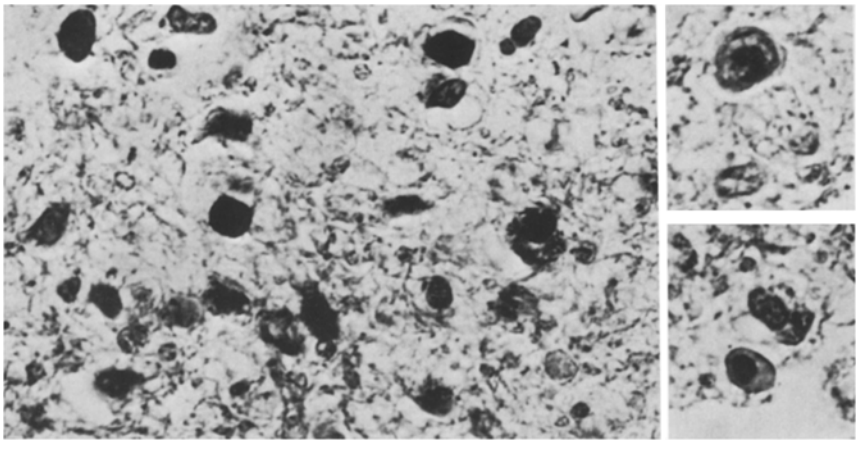

Fig. 3. Intense positive type 1 HSV immunostaining in white matter oligodendrocytes and macrophages in both nuclei and cytoplasm in case 5. Note positive intra-nuclear inclusions (*insets*) (ABC immunoperoxidase + counterstain ×320, ×480)

cytoplasm. In these cells positive nuclear inclusions were more frequent than in neurons (Fig. 3). Staining in both nucleus and processes of astrocytes was prominent in two cases (cases 5 and 6) that showed marked astrocytic reaction (Fig. 4). Perivascular and leptomeningeal lymphocytes were never immunostained in contrast to positive macrophages present in perivascular cuffings or in vascular lumens (Fig. 5).

With regards to variations in anti-HSV1 vs. anti-HSV2 staining, both antibodies equally stained neurons in cases 2, 3, and 5; but neurons in the other cases, oligodendrocytes and macrophages in all the cases were less often immunostained with anti-HSV2. However, astrocytes were more intensely stained when anti-HSV2 was used in cases 5 and 6. In case 4 that was accompanied with severe meningeal symptoms, anti-HSV1 and anti-HSV2 immunoreactivities were similar. In all cases but one (case 1), the white matter was stained less with anti-HSV2 than were cortical areas. The intensity of the immunostaining was weaker with the monoclonal than with the polyclonal antibodies.

As for the positive immunostaining distribution in the brain, the bilateral involvement with a one-side predominance of corroborated neuropathologic data. Also, orbitofrontal lobe, mediobasal temporal lobe, cingulate gyrus, and insula were consistently HSV-positive.

No positive staining was observed in negative controls: PBS controls permitted eliminating non-specific staining due to insufficiently blocked endogenous peroxidase, endogenous biotin, and ABC kit immunoreagents. In two cases (cases 5 and 6) lipofuchsin pigments were observed and easily identified as such because of their dark-brown stain contrasting with the reddish color of AEC substrate; also lipofuchsin remained visible in PBS controls. No positive staining was observed either with irrelevant

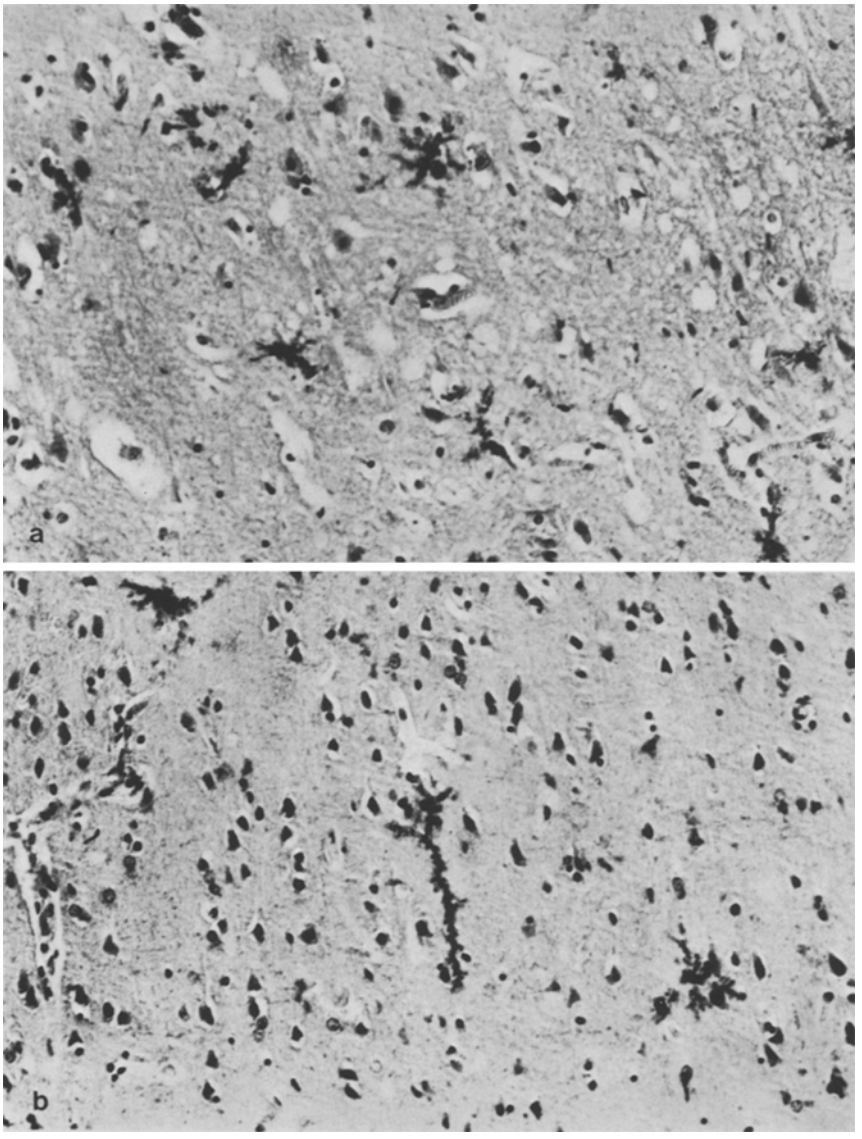

Fig. 4a, b. Positive immunostaining with **a** anti-type 1 HSV and **b** with anti-type 2 HSV in glial cells with prominent staining of astrocyte processes (ABC immuno-peroxidase + counterstain, ×170)

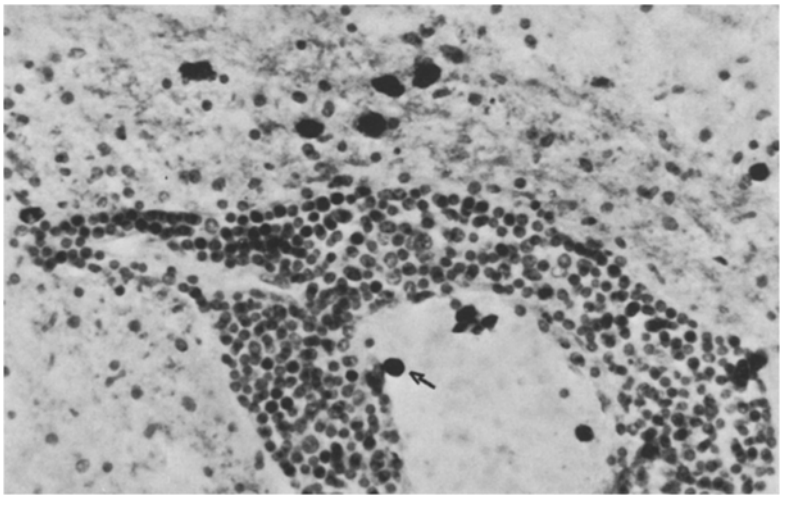

Fig. 5. Perivascular lymphocytes are not immunostained with anti-HSV in contrast to perivascular or intravascular (*arrow*) macrophages (ABC immunoperoxidase + counterstain, ×170)

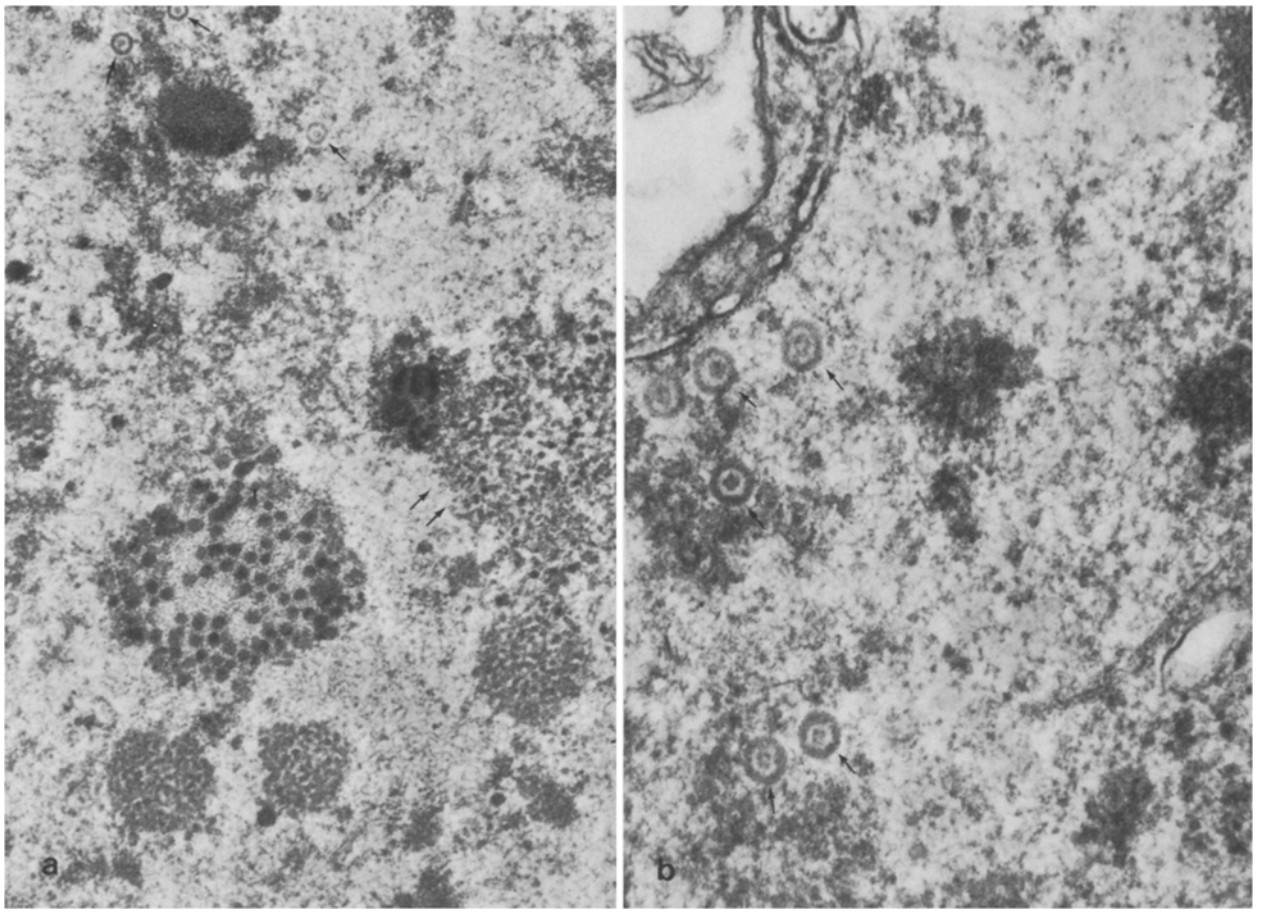

Fig. 6. a Herpes virus particular (*arrows*) and amorphous granular substance aggregates in neuronal nucleus. **b** Virus particles display a characteristic pattern on higher magnification with a central core surrounded by a capsid (× 34,200; × 53,200)

rabbit antiserum or in normal brain tissue obtained from a patient devoid of encephalitis. In one case of non-herpetic encephalitis no positive HSV immunostaining could be demonstrated.

Electron Microscopy

In electron microscopy, viral inclusions consisted in particles with a central core and a capsid displaying a target-like pattern and measuring 850 – 950 Å. They were only in nuclei associated to finely granular matrix or clumps of amorphous intracellular substance (Fig. 6).

Discussion

The diagnosis of HSV encephalitis in humans is based on clinical and neuropathological findings that have already been adequately described in the literature [1, 12, 15, 20, 24]. However, the clue to the diagnosis consists in standard virologic isolation methods or in immunohistochemical demonstration of viral antigens. Needle biopsy of the temporal lobe has previously been advocated as a safe method for HSV encephalitis diagnosis [23]. Indeed, in our case 3 HSV was isolated in such a biopsy. However, the patchy distribution of HSV in tissues is responsible for false negative results [4, 15]. Moreover, HSV is usually not recovered from CSF because of the cell-associated nature of the infection and the presence of pre-existing anti-HSV antibodies [15] and the increase of neutralizing and complement-fixing antibodies in CSF requires up to 3 weeks after the onset of the disease [18].

Immunocytochemical method performed on routine formalin-fixed and paraffin-embedded tissues have already been reported as a reliable method for the demonstration of HSV as the specific agent responsible for the acute encephalitis [4, 9, 10, 14, 16, 17, 26]. This antigen preservation permits the use of this method for retrospective studies on long-term stored histological preparations [4, 10, 17]. Most of the immunohistological studies reported have used immunofluorescence or PAP immunoperoxidase pro-

cedures, but no immunocytochemical study using avidin-biotin-peroxidase complex, known as a more sensitive method [13], have so far been reported for HSV detection. The HSV cell distribution we observed corroborated the findings of others in the major points [4, 10, 16, 26, 27]: (a) prominent cytoplasmic neuronal staining in perikarya and dendrites; (b) neuronal nuclear involvement: in our experience, however, nuclear distribution was inconspicuous in contrast to the findings of other investigators [4, 6]; (c) inconstant staining of intranuclear inclusions; (d) prominent nuclear staining of oligodendrocytes; (e) variable but striking by intense star-like staining pattern of astrocyte processes; (f) positive finely granular or more coarse staining in macrophages; (g) no staining of perivascular or leptomeningeal lymphocytes.

The abundance of the immunostained cells correlated the severity of the inflammatory processes as reported previously [16]. Away from the characteristic areas involved in HSV encephalitis (fronto-orbital lobe, mediobasal temporal lobe, cingulate gyrus, insula) we found, like others, no positive immunostaining [4, 9, 10, 21, 26]. Like in other investigations [16], the immunoreactivity in all specimens sampled 17 years ago did not differ from that of more recently fixed samples.

Type 1 HSV is the commonest non-epidemic fatal encephalitis agent in humans, whereas type 2 HSV is the second commonest venereal disease agent that may cause fatal encephalitis in newborns and various neurologic symptoms in adults [3, 7, 15, 19, 25]. Most of the immunocytochemical studies reported confirmed type 1 HSV involvement in acute necrotizing encephalitis [4, 10, 21, 26]. However, in our experience we observed positive reactions with both type 1 and type 2 HSV antibodies. Because of the questionable specificity of the polyclonal anti-HSV1 and anti-HSV2 due to common antigenic sites [29, 30, 31], the monoclonal antibodies were also used. The immunostaining obtained with the monoclonal antibodies correlated the results obtained with the polyclonal, but the staining intensity was weaker with the monoclonal. These results suggest that both type 1 and 2 HSV or hybrid forms sharing some of the antigenic sites with both types may be involved in encephalitis disorders. However, the immunoperoxidase technique performed on fixed tissues and using commercialized antibodies (although referred to as specific for each type 1 or 2 HSV), lacks of accuracy for HSV typing and may not constitute a reliable and appropriate method, mainly because of probable antigen changes resulting from tissue processing.

Herpes simplex virus are large complex viruses in which the viral DNA is replicated in the nucleus. The capsid proteins are translated in the cytoplasm and transported to the nucleus where the core or nucleocapsid is assembled [2, 15]. The envelopes of herpes viruses are added as the nucleocapsid moves through the nuclear membranes [2, 15]. These data account for viral antigens in both cytoplasm and nuclei as we demonstrated in both cytoplasm and nuclei in infected cells by immunoperoxidase techniques. Herpes virus activation implies integration of viral DNA in a chromosomal DNA (2). This mechanism is possible in mesenchymal cells but unlikely in neurons that do not undergo mitosis and in which DNA integration could take place only during cellular DNA synthesis occuring in cell repair. This may account for the fact that we observed less nuclear staining in neurons than in oligodendrocytes, macrophages, and astrocytes. HSV replication is extremely inefficient since less than 10% of the viral DNA is integrated into virions, and large excesses of proteins are produced. The protein excess accounts for the characteristic nuclear inclusion bodies of HSV infections [8]. Since these inclusions include large amounts of non-structural proteins and consist in finely granular matrix embedding, some bundles of fibrillar structure, and inconstant virus particles in electron microscopy studies [8], it is not surprising that nuclear inclusions are not often immunostained with anti-HSV antibodies: like others [4, 26] we observed only an inconspicuous staining of nuclear inclusions.

Acute HSV encephalitis in humans may represent a primary infection, reinfection, or an activation of latent infection. The disease is particularly remarkable because of its orbitofrontal, mediobasal temporal lobe, cingular gyri, and insula involvement. Inclusions and virions are found in both neurons and glial cells over contiguous anatomic areas suggesting that the virus is spread from cell to cell at the base of the brain within the middle and the anterior fossae [15]. The entry of virus by the olfactory route with subsequent spread along the base of the brain [14] have been suggested on immunofluorescent and electron-microscopic studies that have localized viruses to olfactory nerve, ispsilateral to major temporal involvement [22]. Also infection of olfactory bulbs has been reported in patients who die of herpetic encephalitis [9, 10, 26]. However, this infection of olfactive bulbs is not found uniformly [22]. Alternatively, latency in trigeminal ganglia is recognized, and it may be assumed that encephalitis results from activation and spread from trigeminal ganglia [15]: fibers from trigeminal ganglia (nervus tentorii, meninges medius, and nervus spinosus) innervate structures in anterior and middle fossae, and virus may use this pathway to reach basilar structures [15].

In our study, unfortunately, trigeminal ganglia were never sampled during autopsy, and olfactory

bulbs were often damaged in routinely processed large brain sections. However, when stained (case 1) olfactory bulb displayed positive immunoreactions.

Acknowledgement. We are grateful to T. Bres, F. Fabre, N. Bianco, and S. Reynaud for the preparation of the manuscript.

References

1. Adams JH (1976) Virus diseases of the nervous system. In: Blackwood W, Consellis JAN (eds) Greenfield's neuropathology, 3rd edn. Arnold, London, pp 292–326
2. Aymard M (1983) Le virus herpes simplex. In: L'herpes oculaire. Bulletin des sociétés d'ophtalomologie de France. Rapport Annuel. Boissin, Marseille, pp 19–31
3. Baringer JR (1978) Herpes simplex infections of the nervous system. In: Vinken P, Bruyn GW (eds) Handboock of clinical neurology, vol 34: Infections of the nervous system, pt II. Elsevier, Amsterdam, pp 145–159
4. Budka H, Popow-Kraupp T (1981) Rabies and herpes simplex virus encephalitis. An immunohistological study on site and distribution of viral antigens. Virchows Arch [Pathol Anat] 390:353–364
5. Charpin C, Bhan AK, Zurawski VR, Scully R (1982) Carcinoembryonic antigen (CEA) and carbohydrate determinant (19-9) localization in 121 primary and metastatic ovarian tumors: An immunohistochemical study with the use of monoclonal antibodies. Int J Gynecol Pathol 1:231–245
6. Charpin C, Argemi B, Cannoni M, Oliver C, Gillioz P, Vagneur JP, Toga M (1984) Détection immunocytochimique de calcitonine, d'ACTH, de MSH, de endorphine et de somatostatine dans les carcinomes médullaires de la thyroïde. Etude en immunoperoxidase (PAP, ABC) de 10 cas. Ann Phathol 4:27–35
7. Chastel C (1983) Les différents pouvoirs pathogènes de l'herpes simplex virus dans L'organisme, place de l'oeil dans l'infection herpétique. In: L'herpes oculaire. Bulletin des sociétés d'ophthalmologie de France. Rapport Annuel Boissin, Marseille, pp 33–44
8. Chou SM, Cherry JD (1967) Ultrastructure of Cowdry type A inclusions 1. In human herpes simplex encephalitis. Neurology 17:575–586
9. Dinn JJ (1979) Distribution of herpes simplex virus in acute necrotising encephalitis. J Pathol 129:135–140
10. Esiri M (1982) Herpes simplex encephalitis. J Neurol Sci 54:209–226
11. Hadfield MG, Murray BK, Thomson TA, Young HF (1984) Herpes virus type 1 serum antibodies and brain tumors in humans. Clin Neuropathol 3:68–71
12. Hirano A (1976) Viral infection. In: An outline of neuropathology. Igaku shoin, Tokyo, pp 266–268
13. Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 29:577–580
14. Johnson RT, Mims CA (1968) Pathogenesis of viral infections of the nervous system. N Engl J Med 278:23–30
15. Johnson RT (1982) Viral infections of the nervous system. Herpes virus infections. Raven Press, New York, pp 129–157
16. Kumanishi T, Hirano A (1978) An immunoperoxidase study on herpes simplex virus encephalitis. J Neuropathol Exp Neurol 37:790–795
17. Löhler J (1981) Immunohistochemical demonstration of viral antigens in paraffin embedded autopsy specimens of virally infected central nervous system. Acta Neuropathol [Suppl] (Berl) 7:139–141
18. Mac Callum FO, Chinn IJ, Gostling JVT (1974) Antibodies to herpes simplex virus in the cerebrospinal fluid of patients with herpetic encephalitis. J Med Microbiol 7:325–331
19. Meyer HM, Johnson RT, Crawford IP, Dascomb HE, Rogers NG (1960) Central nervous system syndromes of "viral" actiology. A study of 713 cases. Am J Med 29: 334–347
20. Miller JK, Hesser F, Tompkins VN (1965) Herpes simplex encephalitis. Ann Intern Med 64:93–103
21. Miyake S, Yasuraoka S, Kiryu Y, Satoyoshi E, Kurata T, Aoyama Y (1977) Herpes simplex encephalitis: an autopsy case with isolation of type 1 herpes simplex virus. Acta Neuropathol (Berl) 40:91–93
22. Ojeda VJ (1980) Fetal herpes simplex encephalitis with demonstration of virus in the olfactory pathway. Pathology 12:429–437
23. Olding-Stenkvist E (1974) Acute necrotizing herpes simplex virus encephalitis. Diagnostic problems. Scand J Infect Dis 6:355–360
24. Olson LC, Buescher EL, Artenstein MS, Parkman PD (1967) Herpes virus infections of the human central nervous system. New Engl J Med 277:1271–1277
25. Rappel M, Dubois-Dalcq M, Sprecher S, Thiry L, Lowenthal A, Pele S, Thys JP (1971) Diagnosis and treatment of herpes encephalitis. A multidisciplinary approach. J Neurol Sci 12:443–458
26. Rhodes RH, Novak R, Bealtie JF, West HM, Whetsell WO (1984) Immunoperoxidase demonstration of herpes simplex virus type 1 in the brain of a psychotic patient without history of encephalitis. Clin Neuropathol 3:59–67
27. Swoveland PT, Johnson KP (1979) Enhancement of fluorescent antibody staining of viral antigens in formalin-fixed tissues by trypsin digestion. J Infect Dis 140:758–764
28. Toga M, Tripier MF, Payan H (1969) Encephalite aiguë nécrosante à propos de 4 observations anatomo-cliniques. Ann Pathol 14:165–184
29. Vestergaard BF (1973) Crossed immunoelectrophoretic characterization of herpes virus hominis type 1 and 2 antigens. Acta Pathol Mircobiol Scand [B] 81:808–810
30. Vestergaard BF, Grauballe PC (1977) Crossed immunoelectrophoretic identification of partially purified type common and type-specific herpes simplex virus glycoprotein antigens (39934). Proc Soc Exp Biol Med 156:349–353
31. Vestergaard BF, Grauballe PC (1979) Elisa for herpes simplex virus (HSV) type-specific antibodies in human sera using HSV type 1 and type 2 polyspecific antigens blocked with type heterologous rabbit antibodies. Acta Pathol Microbiol Scand [B] 87:261–263

Received June 24, 1985/Accepted July 25, 1985